IgG Fc glycan and IgG subclass analysis

Jeffrey V. Ravetch * Tineke Cantaert *

Updated date: Jul 1, 2021

*For correspondence: ravetch@rockefeller.edu, tcantaert@pasteur-kh.org

An abbreviated version of this protocol was published in Science in Jun 2021

Antibody fucosylation predicts disease severity in secondary dengue infection

DOI: 10.1126/science.abc7303

Detailed protocol

1. Prepare Protein G-Sepharose resin (Protein G Sepharose 4 Fast Flow (GE Healthcare; 17061804): Wash slurry three times in PBS (without Ca^{2+}/Mg^{2+}) and resuspend 1:1 volume in PBS.
2. Thaw 0.5 ml of plasma sample on ice.
3. Add 0.4 ml PBS containing 2X protease inhibitors (2 Roche Complete tabs (Complete Mini Protease Inhibitor Cocktail (Roche/Sigma 11836153001)/50 ml PBS).
4. If necessary, spin any visible precipitate out with high-speed centrifugation (14,000*g* for 2 min). Keep on ice.
5. Syringe filter (0.22 µm) diluted plasma sample into a microcentrifuge tube.
6. Add 0.2 ml Protein G slurry (from step 1) to each tube and place tubes on end-to-end rotator at 4^{o}C for 60 min.
7. Transfer protein G/plasma slurry to spin column (Mini Bio-Spin Chromatography Columns (BioRad #732-6207)).
8. Remove plasma from spin tube by vacuum or spinning. Wash slurry 5 times with 1 ml PBS. Spec wash flow-through to confirm washing efficiency (A$_{280}$ should be 0).
9. Elute IgG from column with IgG elution buffer (Pierce IgG Elution buffer (Thermo #21004)) into 1.5 ml microcentrifuge tubes containing 1M Tris, pH 9.0 at 10% total volume of eluate (4 fractions of 300 µl elution buffer into separate microcentrifuge tubes i.e. 30 µl 1M Tris in tube + 270 µl IgG elution buffer).
10. Dialyze pooled IgG fractions in PBS. Spec (A$_{280}$ with nanodrop) for IgG concentration.
11. Procced with sample processing for MS analysis of total IgG (see step 17) or continue with isolation of antigen-specific IgGs (Step 12).
12. Add dry agarose beads (NHS-activated agarose beads (Pierce #26197)) to your protein in PBS (300-500 µg protein in 1ml volume for 50mg agarose beads). Incubate at room temperature for 30 min on rotator.
13. Quench active sites by adding equal volume 2 M Tris (pH 7.4). Incubate at room temperature for 30 min.
14. Wash antigen-coupled beads three times in PBS. Divide beads into 1.5 ml microcentrifuge tubes (25 mg beads per 10 samples). Add purified IgG samples to antigen-coupled beads and incubate overnight at 4^{o}C on rotator.
15. Transfer beads/IgG mixture to spin columns (Mini Bio-Spin Chromatography Columns (BioRad #732-6207)).
16. Wash beads 3 times with 1 ml PBS/0.05% Tween followed by 3 times with 1 ml PBS. Resuspend beads in 30-50 µl PBS/0.02% sodium azide. Proceed to step 26 for sample preparation for MS analysis.
17. If sample concentration is known, measure aliquot containing 5-10 µg IgG in a clean 1.5 ml low-bind microcentrifuge tube and dry in Speed-Vap. If possible, avoid starting volumes >25 µl.
18. Add 25 µl 6M guanidine-HCl/10mM dithiothreitol (DTT) in 50 mM Tris-Cl pH8.0. Vortex to mix, spin down, and incubate at 55-60^{o}C for 1 hour.
19. Cool to room temperature and add freshly prepared iodoacetamide in Tris-Cl pH8.0 to 60 mM, vortex to mix, spin down, and incubate in dark at room temperature for 1 hour.
20. Add DTT in water to 30 mM, vortex to mix, spin down, and incubate at room temperature for 15 min.
21. Add 50 mM ammonium bicarbonate in water so total sample volume is > 150 µl.
22. Add 0.5 µg MS-grade trypsin in water, vortex to mix, spin down and incubate overnight at 37^{o}C.
23. Allow digests to cool, spin down, and stop digests by adding 5 µl formic acid (FA). Add trifluoroacetic acid (TFA) in water to 0.1% (v/v) and proceed with reverse phase solid phase extraction (RP-SPE) using C18 or equivalent functional group and manufacturer's recommended procedure for protein digest clean-up.
24. Dry collected eluate in Speed-Vap.
25. Reconstitute in 0.1% formic acid (FA) (v/v) for analysis by mass spec (step 30).
26. Spin down samples and remove as much storage buffer as possible.
27. Add 100 µl Milli-Q water, spin down, and remove as much solvent as possible.
28. Dry in Speed-Vap and proceed with trypsin digestion and RP-SPE as in steps 18-24.
29. Filter final sample for injection in 0.1% FA through a 0.22 µm regenerated cellulose or cellulose acetate filter prior to analysis.
30. NanoLC-MS/MS analysis is performed on tryptic peptides containing the N279 glycan using an UltiMate3000 nanoLC (Dionex) coupled with a hybrid triple quadrupole linear ion trap mass spectrometer, the 4000 Q Trap (SCIEX). Data are acquired using Analyst 1.6.1 software (SCIEX) for precursor ion scan triggered information dependent acquisition (IDA) analysis for initial discovery-based identification.
31. For quantitative analysis of the glycoforms at the N297 site across the three IgG subclasses (IgG1, IgG2 and IgG3/G4), multiple-reaction monitoring (MRM) analysis for selected target glycopeptides, is applied using the nanoLC-4000 Q Trap platform to the samples after trypsin digestion. The m/z of 4-charged ions for all different glycoforms of the core peptides from three different subclasses as Q1 and the fragment ion at m/z 366.1 as Q3 for each of transition pairs are used for MRM assays. A native IgG tryptic peptide (131-GTLVTVSSASTK-142) with transition pair of, m/z 575.9+2 to mz 780.4 (y8+) is used as a reference peptide for normalization purposes.
32. All raw MRM data are processed using MultiQuant 2.1.1 (SCIEX). MRM peak areas are automatically integrated and manually inspected. In the event that automatic peak integration by MultiQuant fails, manual integration is performed using the MultiQuant software.

How to cite:（Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used）

1. Ravetch, J. V. and Cantaert, T. (2021). IgG Fc glycan and IgG subclass analysis. Bio-protocol Preprint. bio-protocol.org/prep1236.
2. Bournazos, S., Vo, H. T. M., Duong, V., Auerswald, H., Ly, S., Sakuntabhai, A., Dussart, P., Cantaert, T. and Ravetch, J. V.(2021). Antibody fucosylation predicts disease severity in secondary dengue infection. Science 372(6546). DOI: 10.1126/science.abc7303

Copyright: Content may be subjected to copyright.